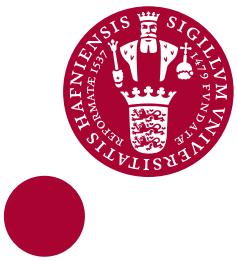

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RESEARCH PAPER

Interactive effects of elevated CO_2, warming, and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem

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Abstract

Global change factors affect plant carbon uptake in concert. In order to investigate the response directions and potential interactive effects, and to understand the underlying mechanisms, multifactor experiments are needed. The focus of this study was on the photosynthetic response to elevated CO_2 [CO2; free air CO_2 enrichment (FACE)], drought (D; water-excluding curtains), and night-time warming (T; infrared-reflective curtains) in a temperate heath. A/C_i curves were measured, allowing analysis of light-saturated net photosynthesis (P_n), light- and CO_2-saturated net photosynthesis (P_{max}), stomatal conductance (g_s), the maximal rate of Rubisco carboxylation (V_{cmax}), and the maximal rate of ribulose bisphosphate (RuBP) regeneration (J_{max}) along with leaf $\delta^{13}C$, and carbon and nitrogen concentration on a monthly basis in the grass *Deschampsia flexuosa*. Seasonal drought reduced P_n via g_s, but severe (experimental) drought decreased P_n via a reduction in photosynthetic capacity (P_{max}, J_{max}, and V_{cmax}). The effects were completely reversed by rewetting and stimulated P_n via photosynthetic capacity stimulation. Warming increased early and late season P_n via higher P_{max} and J_{max}. Elevated CO_2 did not decrease g_s, but stimulated P_n via increased C_i. The T×CO2 synergistically increased plant carbon uptake via photosynthetic capacity up-regulation in early season and by better access to water after rewetting. The effects of the combination of drought and elevated CO_2 depended on soil water availability, with additive effects when the soil water content was low and D×CO2 synergistic stimulation of P_n after rewetting. The photosynthetic responses appeared to be highly influenced by growth pattern. The grass has opportunistic water consumption, and a biphasic growth pattern allowing for leaf dieback at low soil water availability followed by rapid re-growth of active leaves when rewetted and possibly a large resource allocation capability mediated by the rhizome. This growth characteristic allowed for the photosynthetic capacity up-regulations that mediated the T×CO2 and D×CO2 synergistic effects on photosynthesis. These are clearly advantageous characteristics when exposed to climate changes. In conclusion, after 1 year of experimentation, the limitations by low soil water availability and stimulation in early and late season by warming clearly structure and interact with the photosynthetic response to elevated CO_2 in this grassland species.

Key words: CLIMAITE, climate change, FACE, grassland, leaf $\delta^{13}C$, multifactor experiment, stomatal conductance, water use efficiency.

Abbreviations: C, leaf carbon concentration; C_i, intercellular CO_2 concentration; $\delta^{13}C$, carbon isotope ratio; D, experimental drought; CO2, experimental elevated CO_2, g_s, stomatal conductance; J_{max}, maximal rate of RuBP regeneration;N, leaf nitrogen concentration; P_{max}, maximal light- and CO_2-saturated photosynthesis; P_n, light-saturated net photosynthesis; SWC, soil water content; T, passive night-time warming; T_r, transpiration; V_{cmax}, maximal rate of Rubisco carboxylation; WUE (=P_n/T_r), water use efficiency.

Introduction

Environmental changes caused by increased emissions of greenhouse gasses have been predicted to influence the stability of ecosystems worldwide (IPCC, 2007). For terrestrial plants and ecosystems, increases in the atmospheric CO_2 concentration and air temperature as well as changes in precipitation patterns are expected to have strong impacts on the carbon balance. Regional climate models predict enhanced temperature and a change in the pattern of precipitation in Denmark, resulting in longer summer drought periods and the occasional incidences of high amounts of precipitation (Christensen and Christensen, 2003). Effects of environmental changes such as elevated CO_2, temperature, and precipitation on plants and ecosystems have primarily been investigated as effects of a single factor or two factors in combination (e.g. Beier *et al.*, 2004; for an overview see Rustad, 2008). Since all environmental changes occur concurrently, in order to investigate potential interactions between factors, it is important to study the effects in response to the factors alone and when combined.

Warming generally increases net primary production (Rustad *et al.*, 2001; Shaw *et al.*, 2002; Dukes *et al.*, 2005; Penuelas *et al.*, 2007), with the strongest responses on sites with low aridity (Penuelas *et al.*, 2007). On the leaf level, increased daytime temperature may increase light-saturated net photosynthesis (P_n), especially during cold periods (Sage and Kubien, 2007). Plant respiration in response to warming probably affects the plant carbon uptake. During the day, respiration increases with temperature and decreases the plant net carbon uptake (Atkin and Tjoelker, 2003), whereas during the night-time increased plant respiration can stimulate carbon sink strength and daytime P_n (Turnbull *et al.*, 2002, 2004). Indirect effects of warming can increase growing season length (Menzel and Fabian, 1999; Walther *et al.*, 2002; Cleland *et al.*, 2006), change phenology (Harte and Shaw, 1995; Wan *et al.*, 2005; Menzel *et al.*, 2006), increase soil nitrogen mineralization and availability (Rustad *et al.*, 2001), reduce the soil water content (SWC) (Volk *et al.*, 2000; Morgan *et al.*, 2004; Wan *et al.*, 2005), and affect species composition and community structure (Shaver *et al.*, 2000; Wan *et al.*, 2005). The indirect effects have been argued to be more important than the direct effects (Körner, 2000; Shaver *et al.*, 2000; Morgan *et al.*, 2004; Lou *et al.*, 2007).

In response to mild to moderate drought, stomatal conductance (g_s) reduction decreases transpiration (T_r) and P_n. More intensive drought induces the down-regulation of light- and CO_2-saturated net photosynthesis (P_{max}), the maximal rate of Rubisco carboxylation (V_{cmax}), and the maximal rate of ribulose bisphosphate (RuBP) regeneration (J_{max}); a reduced content of Rubisco decreases P_n together with g_s, and mesophyll conductance reduction dominates during severe drought (Flexas and Medrano, 2002; Penuelas *et al.*, 2007).

Elevated CO_2 predominantly decreases g_s, stimulates P_n, and increases plant water use efficiency (WUE) (Curtis, 1996; Curtis and Wang, 1998; Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). At elevated CO_2, V_{cmax} is often reduced through reduction of Rubisco content and thereby nitrogen investment (Drake *et al.*, 1997; Parry *et al.*, 2003). This response leads to a down-regulation response with decreased leaf nitrogen, increased leaf C/N ratio, and reduced J_{max} and P_{max} found in many studies (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). The magnitude of the response of P_n to elevated CO_2 is modulated by nitrogen supply and the constraints of the capacity to utilize photosynthates (Ainsworth and Rogers, 2007). The importance of improved water relations via reduced water consumption in elevated CO_2, which to some degree sustain P_n in dry periods, has been stressed in particular in grasslands and forest exposed to elevated CO_2 (Körner, 2000; Volk *et al.*, 2000; Medlyn *et al.*, 2001; Morgan *et al.*, 2004; Leuzinger and Körner, 2008). During dry periods, the combined responses of leaf area index (LAI) and leaf g_s determine the plant water consumption. Therefore, water status may strongly co-determine plant biomass responses to CO_2 enrichment (Acock and Allen, 1985; Gifford, 1992; Koch and Mooney, 1996; Fredeen *et al.*, 1997; Field *et al.*, 1997; Niklaus *et al.*, 1998).

This study is part of the CLIMAITE experiment (Mikkelsen *et al.*, 2008), where treatments of passive night-time warming, elevated CO_2, and periods of drought are applied alone and in all combinations. To investigate the photosynthetic responses of the dominant grass *Deschampsia flexuosa*, monthly campaigns of leaf gas exchange measurements (A/C_i curves) were performed, and leaf characteristics of carbon, nitrogen, and ^{13}C natural abundance ($\delta^{13}C$) were analysed. The values of $\delta^{13}C$ represent an integrated measure of WUE over time (Farquhar *et al.*, 1982). It was assumed that the measured $\delta^{13}C$ values were the product of g_s reductions over time but that they would also be influenced by a higher leaf C_i/C_a ratio, mediated by reduction in photosynthetic capacity measures (J_{max}, V_{cmax}, and P_{max}). The measurements made allowed for analysis of the accumulated responses of carbon uptake, water status, and nitrogen balance in the experimental system. The impacts of the three environmental factors alone and in combination were evaluated to elucidate possible shifts in importance of the treatments during the growing season.

It was hypothesized that:

(i) Elevated CO_2 (CO2) would increase P_n via increased C_i despite acclimation with g_s reduction and photosynthetic capacity (probed with P_{max}, J_{max}, and V_{cmax}) down-regulation.

(ii) Elevated CO_2 would sustain P_n in dry periods via soil water savings.

(iii) Passive night-time warming (T) would increase P_n and decrease $\delta^{13}C$ in spring via earlier maturation of photosynthetic capacity and in autumn via delayed senescence maintaining high photosynthetic capacity.

(iv) Summer drought (D) would decrease P_n via g_s reduction and only decrease P_n via photosynthetic capacity down-regulation under severe water shortage.

(v) Responses to combinations of T, D, and CO2 would mainly be additive, but with potential for interactive effects.

Materials and methods

Site and experimental set-up

The experimental site is a dry heathland ecosystem in North Zealand, Denmark, dominated by the C_3 grass *D. flexuosa* (L.) Trin and the evergreen dwarf shrub *Calluna vulgaris* (L.) Hull. The experimental treatments were elevated CO_2 (CO2), passive nighttime warming (T), summer drought (D), all combinations, TD, TCO2, DCO2, and TDCO2, and unmanipulated control (A), replicated in six blocks in a split-plot design. Each block consisted of two 6.8 m diameter octagons, each divided into four plots. In one octagon the CO_2 concentration was elevated to 510 ppm during the daytime with the FACE technique (free air CO_2 enrichment; Miglietta *et al.*, 2001) and in the other octagon the CO_2 level was ambient. In each octagon, automated curtains covered one half (two plots) during the night, preserving a proportion of the daily incoming radiation energy, which increased the night air temperature by up to 4 °C, on average 1–2 °C. Perpendicular to the infrared (IR)-reflective curtains was a rain-excluding curtain which automatically was activated by rain during intended drought periods. In each experimental plot the soil temperature was measured continuously at 2 cm and 10 cm depth, and the SWC at 0–20 cm and 0–60 cm depth. Two climatic stations recorded precipitation, air temperature, photosynthetically active radiation, and wind speed and direction. The CO_2 and warming treatments were initiated on 3 October 2005. A drought period was initiated on 3 July 2006 and continued until 4 August when the SWC reached ~5% in the top 20 cm of the soil. For further description of the site and experimental set-up, see Mikkelsen *et al.* (2008).

Leaf gas exchange

CO_2 and H_2O leaf gas exchange measurements were conducted *in situ* by using two LI-6400s (LI-COR Biosciences, Lincoln, NE, USA) connected to standard 2×3 cm chambers with light-emitting diode (LED) light sources (6400-02B). They were carried out in the periods 11–18 May, 12–16 June, 10–13 July, 14–21August, 11–22 September, and 9–20 October, 2006. During each campaign, measurements were conducted on one leaf sample in each experimental plot (48 plots) and the resulting treatment replications were n=6. Only fully expanded, healthy leaves from the top of the vegetation were selected. Pilot studies resulted in the following methodology securing highly reproducible measurements: 10–20 *D. flexuosa* leaves were positioned side by side and gently fixed by hairgrips during each of the monthly periods of measurements. The leaf cuvettes were fixed to the samples during measurements using flexible arms (Magic Arm 143, Manfrotto, Italy). During the following harvest, care was taken to ensure that area and weight were determined on exactly the same material as inserted in the leaf cuvette.

Samples were acclimated for 2–4 min at ambient CO_2 (380 ppm), until net photosynthesis and stomatal conductance were stabilized [±1 coefficient of variation (CV) over 30 s]. The CO_2 response curves were measured by stepping CO_2 down from 380 ppm to 300, 200, 100, and 50 ppm and then back to 380 ppm for 4 min re-acclimation, until the initial state was reached again. Then CO_2 was then stepped up to saturation from 380 ppm to 450, 510, 650, 800, 1000, 1200, and 1400 ppm at the saturating light level of 1500 μmol photons m^{-2} s^{-1}. The LI-COR Auto program 'A/C_i-curve' was used (settings: minimum 45 s and maximum 60 s, reference CO_2 stable in 10 s with CV <1%, C_i stable in 10 s with CV <1 %, IRGA matching performed at each step). Block temperature was held constant at 25 °C and sample relative humidity was stabilized at 45–55% during measurements. All measurements were area corrected. Leak corrections was applied with the empty chamber approach (Manter and Kerrigan, 2004). Gas exchange parameters, such as P_n, T_r, WUE (=P_n/T_r), g_s, and the C_i, were extracted from the CO_2 response curves at the reference CO_2 level (380 ppm CO_2 in non-FACE plots and 510 ppm CO_2 in FACE plots). This allowed calculation of the C_i/C_a with C_a set to 380 ppm in ambient CO_2 and 510 ppm in elevated CO_2. P_{max} was extracted at 1200 ppm CO_2. Calculation of V_{cmax} and J_{max} followed the approach of Dubois *et al.* (2007) after Farquhar *et al.* (1980).

Leaf weight, area, nitrogen, carbon, and $\delta^{13}C$

Immediately after harvest, digital pictures were taken of the leaves flattened by transparent acrylic plates beside a quadrant of known area. The photographs were converted to black and white and loaded into a pixel-counting program (Bitmap, S. Danbæk, Department of Biology, University of Copenhagen), by which the leaf areas were determined. Then the fresh weight was determined. The dry weight was determined after oven drying at 80 °C for 48 h. The plant material was analysed for C and N concentration and $\delta^{13}C$ with an elemental analyser (EuroVector, Milano, Italy) coupled to an IsoPrime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). During analysis, the internal reference gas was calibrated against certified standards from the International Atomic Energy Agency, and plant material calibrated against certified standards was used as a working standard. From the dry weight and leaf area, the specific leaf area (SLA) was calculated. Leaf water content was calculated from fresh and dry weights.

Statistics

Analyses of variance were performed on linear mixed effects models with the following fixed effects: month, T, D, and CO2, and their interactions T×D, T×CO2, D×CO2, and T×D×CO2, using proc mixed in SAS (Statistical Analysis Systems Institute, 2009). The split-plot design of the experiment was described by including octagon (O), O×T, and O×D as random factors. Pretreatment data were initially included as covariates as fixed factors. The full model was reduced by backward selection until factors with P <0.1 remained. The denominator degrees of freedom were corrected according to the Satherwaite procedure. Significance levels are reported in the figures and tables as a tendency with † when P <0.10 and as significant with * when P <0.05, ** when P<0.001, and *** when P <0.0001. All data were tested for and confirmed to be normally distributed; some response data were transformed to fulfil the assumption of homogeneity of variance. To interpret the direction of the significant responses, the group least square means were compared.

Results

Environmental conditions

In 2006, the last snow melted in late March and from early April the mean daily temperature (at 2 m height) gradually increased from 0 °C to 25 °C in late July; 2006 was the warmest year ever recorded in Denmark, 9.4 °C (www.DMI.dk), and mean daily air temperatures were >11 °C in late November. The warming treatment increased the 24 h mean daily temperature by 1–2 °C and

there was a 33% higher number of accumulated growing degree days (GDDs) from 1 April to 15 May and an annual 7% increase in GDDs in warmed plots compared with controls (Mikkelsen *et al.*, 2008). The warming treatment increased the maximum temperatures in the late night/early morning in both the air and the soil, but the effect was gradually reduced during the day. In the air, this effect of the warming treatment occasionally lasted 3–5 h after sunrise, while in the soil the warming effect was less dynamic and some warming was sustained throughout the day. (Mikkelsen *et al.*, 2008). However, the average air temperature increased in response to warming only during the night-time, and there were no effects on average daytime temperature (Fig. 1). The concentrations of CO_2 in the FACE octagons were close to 510 ppm on an houly basis during the daytime (Mikkelsen *et al.*, 2008), but there were a large variation on a smaller time scale just as experienced in all FACE studies. The control plots were not affected by the CO_2 in the FACE octagons.

SWC fluctuated with incoming precipitation, ranging from ~5% to 25% at 0–20 cm and 0–60 cm depth, and the SWC at 0–20 cm decreased from 17.2% in April to 8.8% in July in the ambient plots (Figs 2, 3). This natural low SWC was extended by the experimental drought period (D) in which SWC decreased even further towards August. In this period the non-drought plots benefitted from some incidents of precipitations. Rewetting increased the SWC at 0–20 cm to 18% in August, 13.6% in September, and 16.5% in October in the controls. SWCs were significantly decreased in the warming treatments in May, June, August, and September and in response to the experimental drought in July (Figs 2, 3). There was a significant interaction effect of D×CO2 on SWC at 0–60 cm in the experimental drought period (Fig. 2) and on SWC at 0–20 cm during the leaf gas exchange campaign in July (Fig. 3). A D×CO2 interaction revealed that the combination of elevated CO_2 and experimental drought compensated for the negative effects of drought by keeping soil moisture levels marginally higher in TDCO2 and DCO2 compared with the TD and D treatments (Figs 2, 3).

Seasonal changes in leaf gas exchange

P_n generally showed high levels across treatments in May (range 8–16 μmol CO_2 m^{-2} s^{-1}), lower levels during June, July, August, and September (range 4–13 μmol CO_2 m^{-2} s^{-1}), and again high levels in October (range from 6 μmol CO_2 m^{-2} s^{-1} to 11.5 μmol CO_2 m^{-2} s^{-1}) (Fig. 4). g_s levels in the control plots decreased during May, June, and to July from 0.116 to 0.077 and 0.066 mmol H_2O m^{-2} s^{-1}. In August the g_s in the controls was again high (0.195 mmol H_2O m^{-2} s^{-1}). It dropped in September to 0.090 mmol H_2O m^{-2} s^{-1} and was intermediate in October, 0.132 mmol H_2O m^{-2} s^{-1} (Fig. 5). The WUE across season varied from ~3 μmol CO_2 mmol H_2O^{-1} to 9 μmol CO_2 mmol H_2O^{-1}, with a seasonal low in August and the highest values in May and October (Table 1). When including the clear effect of month (P <0.0001), elevated CO_2 significantly increased P_n, g_s, and WUE (all P <0.0001) across seasons.

Treatment responses in leaf gas exchange

P_n was higher in the TCO2 and TDCO2 treatments, as compared with T or CO2 alone, leading to a significant T×CO2 interaction in May, June, and August (Fig. 4). Elevated CO_2 increased P_n in June, July, and October. Drought decreased P_n in July and August, but after rewetting the P_n was increased in the previously drought-treated plots in September. In September the D and CO2 in combination synergistically increased P_n (Fig. 4).

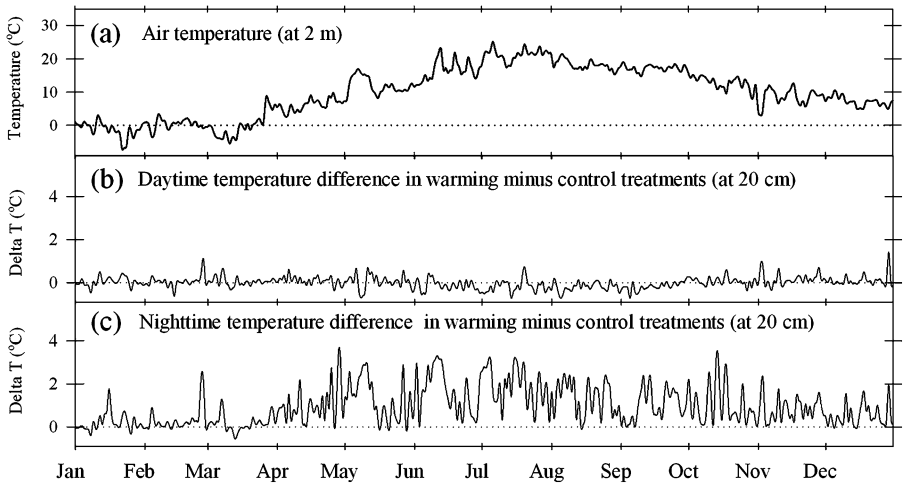

Fig. 1. Temperature. Daytime air temperature at 2 m height (hourly means) at the experimental site is depicted as a black line and the 0 °C reference as a dotted line. (b) Daytime temperature difference at 20 cm height for the warming minus control treatments. No significant effects on daytime temperature appeared; thus, temperature was not directly affected when leaf gas exchange and fluorescence measurements were conducted. (c) Night-time temperature difference at 20 cm height for warming minus control treatments. Night-time corresponds to the period where the passive night-time warming treatment was active.

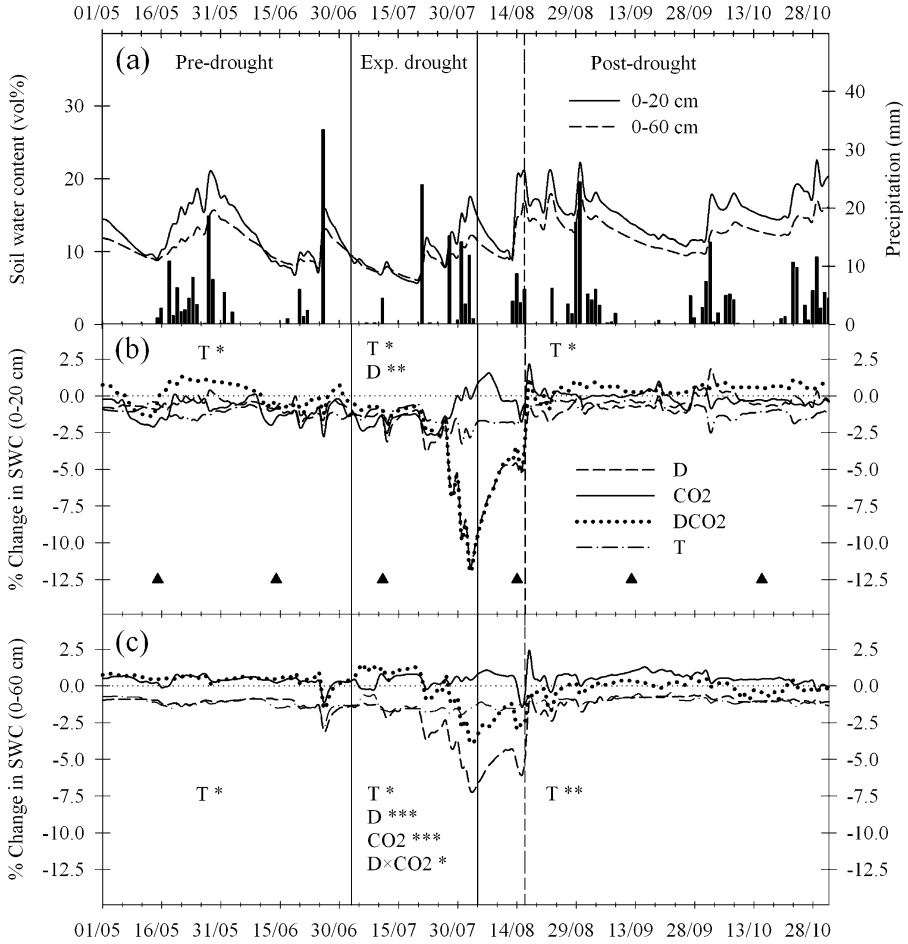

Fig. 2. Precipitation and soil water content. Shown are: (a) the daily averages of soil water content in control plots at 0–20 cm and 0–60 cm depth and the daily accumulated precipitation. The panel is divided into pre-drought, experimental drought, and post-drought by vertical black lines. The vertical dashed line indicates a lag phase after the end of the experimental drought period due to sparse precipitation. (b) Change in percentage of the soil water content at 0–20 cm with treatment compared with control. The dotted line is the zero reference line for the unmanipulated control. The treatments are: elevated CO_2 (CO2); experimental drought (D); passive night-time warming (T); and the combination of DCO2. Warming and elevated CO_2 treatments were active in all periods, whereas the drought was only active in the experimental drought period. All treatments were replicated six times, in total 48 plots. In each period the significant effects of the experimental factors T, D, CO2, and their interactions are indicated with *** for P <0.001, ** for P <0.01, and * for 0.01<P<0.05. The timings of measurement surveys are indicated with triangles in a horizontal line at –12.5%. (c) Change in percentage of the soil water content at 0–60 cm with treatment compared with control.

g_s was increased in response to elevated CO_2 in June and October (Fig. 5). In the TCO2 treatments, a T×CO2 interaction synergistically increased g_s in June. Drought decreased g_s in July and August, but after rewetting, g_s increased in the previously drought-treated plots in September. WUE increased in elevated CO_2 in May, July, September, and October (Table 1). Warming increased WUE in May and a T×CO2 interaction caused a synergistic increase in WUE in August. Intercellular CO_2 was increased in elevated CO_2 in all months, increased by warming in May and August, and a T×CO2 effect synergistically increased C_i in TCO2 and TDCO2 treatments in August and October (Table 1).

P_{max} was significantly increased in response to warming in May and October (Fig. 6). Elevated CO_2 decreased P_{max} in June (Fig. 6). In the TCO2 and TDCO2 treatments a T×CO2 effect synergistically increased P_{max} in June, but this was reversed in July where T×CO2 synergistically decreased P_{max}. Drought decreased P_{max} in the experimental drought period in July and beyond it into August, but after rewetting in September and October the P_{max} increased in the experimental drought plots. In DCO2 and TDCO2, a D×CO2 interaction synergistically increased P_{max} in September and October (Fig. 6).

J_{max} demonstrated similar effects to P_{max}, except that elevated CO_2 was not observed to reduce J_{max} in September (Table 1). The V_{cmax} was lowered in elevated CO_2 in most months (June, July, August, and September). Experimental drought reduced V_{cmax} (August), but rewetting increased V_{cmax} in the former drought plots (September). In TCO2 and TDCO2, a T×CO2 interaction synergistically decreased V_{cmax} in July (Table 1).

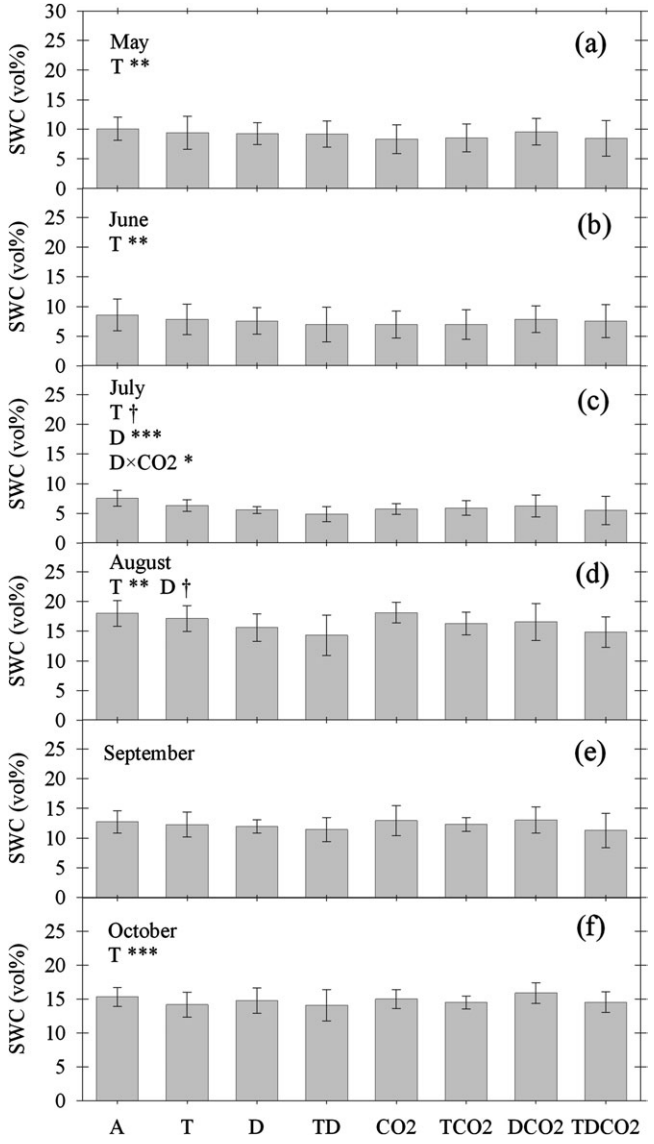

Fig. 3. Soil water content at 0–20 cm depth during each gas exchange campaign. Shown are the mean ±SE (n=6). Treatments are: A, unmanipulated control; T, passive night-time warming; D, experimental drought, active in July to early August; TD, warming and drought combined; CO2, elevated CO_2; TCO2, warming and elevated CO_2 combined; DCO2, drought and elevated CO_2 combined; and TDCO2, warming, drought, and elevated CO_2 combined. Statistically significant effects of single factors (T, D, and CO2) and interactions (T×CO2 and D×CO2) are shown with treatment letters (*** if P <0.0001, ** if P <0.001, * if P <0.05 and † if P <0.10).

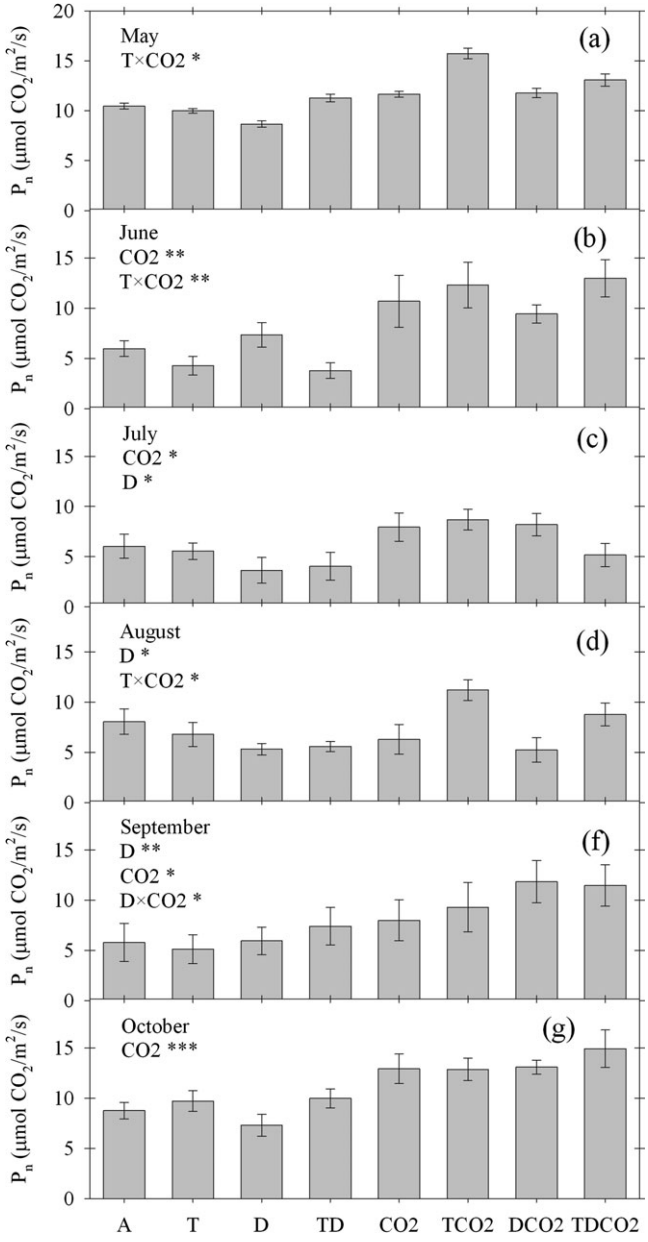

Fig. 4. Light-saturated net photosynthesis at treatment CO_2, P_n. Shown are the mean ±SE. Treatment abbreviations and statistics are as in Fig. 3.

Leaf characteristics

In all treatments the leaf characteristics of *Deschampsia* were related to the biphasic growth pattern, with two peaks of vegetative green biomass closely linked to the SWC. The first phase (May, June, and July) was characterized by biomass increments in May and June during ample SWC and a large dieback in July along with declining water availability. The second leaf phase (August, September, and October) was initiated by rewetting and was characterized by the leaves having a high nitrogen content and a balanced C/N ratio in contrast to the nitrogen dilution during first phase. The leaf C/N ratio increased in elevated CO_2 in May, June, July, and September (Fig. 7). Drought decreased the C/N ratio in September, while the interaction detected (D×CO2) in October meant that the increased C/N ratio in elevated CO_2 did not occur when combined with drought. In general, the changes in the C/N ratios were governed by differences in nitrogen concentrations (Fig. 7).

Leaf water content increased in elevated CO_2 in May, June, and September (P <0.006, P <0.009, and P <0.002, data not shown) and decreased in drought in July and August (P <0.04 and P <0.0001, data not shown). There were no significant effects on SLA. Leaf $\delta^{13}C$ was decreased, in all months, under elevated CO_2, as a result of

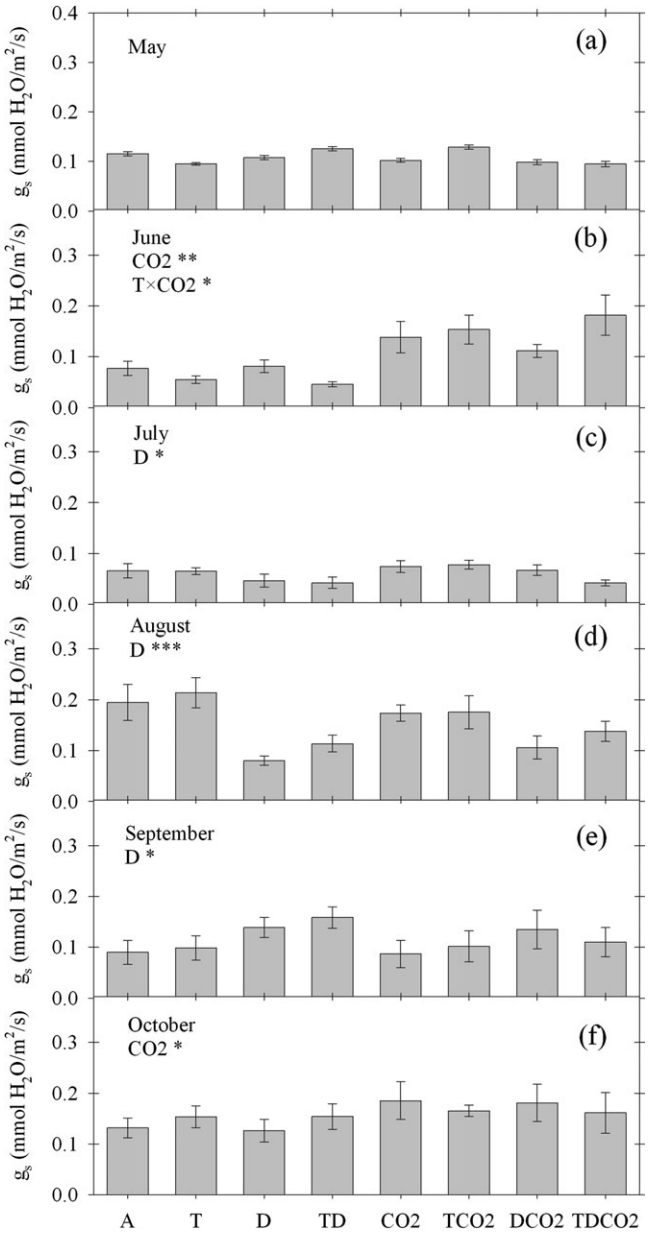

Fig. 5. Leaf stomatal conductance at treatment CO_2, g_s. Shown are the mean ±SE. Treatments abbreviation and statistics are as in Fig. 3.

uptake of the lower $\delta^{13}C$ signature of the industrial CO_2 gas (average air $\delta^{13}C$ during the primary and secondary leaf phase in the elevated CO_2 treatment was –10.6‰ and –13.8‰, respectively, as opposed to –8‰ in ambient air) added to the FACE plots (Fig. 8). Warming decreased the $\delta^{13}C$ in August, September, and October, and a T×CO2 interaction was significant in May, June, and August due to a decrease in $\delta^{13}C$ only when warming and elevated CO_2 were combined. The interaction D×CO2 was significant in July due to an increase in $\delta^{13}C$ in response to drought only when combined with elevated CO_2 (Fig. 8).

The slopes of regressions between leaf $\delta^{13}C$ and WUE were negative in both elevated CO_2 and ambient CO_2 (P <0.05, Fig. 9a). Correcting $\delta^{13}C$ for the difference in air $\delta^{13}C$ signatures between phases in elevated CO_2 resulted in similar linear regression slopes (α) for the $\delta^{13}C$ versus WUE relationship in elevated and ambient CO_2 (α= –0.29). Lower soil water conditions occurred during the primary phase compared with the secondary leaf phase, and the leaf $\delta^{13}C$ did not vary significantly with SWC at 0–20 cm or 0–60 cm across treatments (Fig. 9b, d). The relationships between leaf $\delta^{13}C$ and C_i/C_a were positive, with higher α in elevated compared with ambient CO_2 (Fig. 9c).

Discussion

Responses to elevated CO_2

When water availability was declining (May–July), the C/N ratio was higher in the elevated CO_2 treatment, indicating that some of the additional assimilated carbon was allocated to leaf growth without a corresponding allocation of nitrogen. As some level of soil moisture is necessary for the mineralization processes, this probably influenced the C/N ratios. In the longer term, nitrogen limitation very probably constrains the potential for increased productivity in response to CO_2 (Finzi *et al.*, 2002; Lou *et al.*, 2004; Hungate *et al.*, 2006; Reich *et al.*, 2006), but in this dry ecosystem it may be difficult to separate this effect from the effect of the ample water conditions.

The P_n was higher in elevated CO_2, mainly driven by higher C_i during most of the growing season in accordance with the first hypothesis proposed here. Photosynthetic capacity regulations were dynamic, but down-regulation took place with V_{cmax} reduction in most months, whereas P_{max} and J_{max} responses were more stable and only declined in the second leaf phase after rewetting. These responses are consistent with most findings on photosynthetic responses to elevated CO_2 (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Leaky *et al.*, 2009). The WUE was higher in elevated CO_2, but not via g_s reduction even in the driest month, July, and therefore the higher WUE in this study was caused by a higher P_n which is in contrast to several other studies with other species (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007).

In contrast to the first hypothesis proposed here, no general reduction in g_s was seen under elevated CO_2. Complex g_s responses have been reported, and are purported to depend on the degree of drought and the species-specific water demand; the g_s net response can be either positive, negative, or zero, owing to the antagonistic nature of direct (reducing) and indirect (enhancing) CO_2 effects on g_s (Knapp *et al.*, 1996; Volk *et al.*, 2000). In the present study the observed g_s values were actually higher in the elevated CO_2 treatment, along with P_{max} and J_{max} up-regulation, which in combination increased P_n in June. This demonstrates the dynamic capability of the photosynthetic capacity in *Deschampsia*, which may be related to the peak of the first phase of leaf growth and in combination with the relatively high June SWC level (10–15%). Further, $\delta^{13}C$, an integrating measure of the response of WUE, did not vary significantly with SWC, but instead with C_i/C_a. This indicates

Table 1. For each month the F-values and significance levels (*P <0.05; ** P <0.01; *** P <0.0001) for the main effects night-time warming (T), drought (D), elevated CO_2 (CO2), and their interactions on light-saturated net photosyntheis (P_n), stomatal conductance (g_s), water use efficiency (WUE), maximal light- and CO_2-saturated net photosynthesis (P_{max}), maximal RuBP regeneration rate (J_{max}), maximal rate of Rubisco carboxylation (V_{cmax}), leaf carbon to nitrogen ratio (C/N), and leaf $\delta^{13}C$ by a linear mixed model ANOVA

Degrees of freedom (df), numerator (Num), denominator (Denom), not detected (ND) increase (↑), decrease (↓), synergistic increase (↑↑), synergistic decrease (↓↓).

Deschampsia flexuosa		**T**			**D**			**CO2**			**T×D**	**T×CO2**			**D×CO2**			**T×D×CO2**
Num df		1			1			1			1	1			1			1
Denom df		10			10			5			10	10			10			10
P_n	May	1.27			0.14			0.24			0.03	5.83	*	↑↑	0.05			1.37
	June	0.09			0.00			12.54	**	↑	0.01	10.81	**	↑↑	0.47			0.94
	July	0.31			5.15	*	↓	8.65	**	↑	0.98	0.56			0.01			3.80
	August	2.62			4.82	*	↓	2.57			0.09	5.02	*	↑↑	0.80			1.66
	September	0.18			8.47	**	↑	6.24	*	↑	2.63	0.01			13.41	**		1.08
	October	2.06			0.83			32.84	***	↑	0.29	0.72			0.96			0.01
g_s	May	0.00			0.03			0.04			0.03	0.07			0.08			2.37
	June	0.03			0.25			19.03	**	↑	0.13	8.85	*	↑↑	0.21			0.79
	July	0.45			5.97	*	↓	0.98			1.50	0.66			0.05			1.78
	August	0.79			9.79	**	↓	0.01			0.26	0.06			1.21			0.04
	September	2.26			9.52	**	↑	0.14			0.01	2.19			0.01			4.83
	October	0.04			0.07			18.24	**	↑	0.03	2.47			0.01			0.10
WUE	May	5.46	*	↑	0.45			20.05	**	↑	0.31	0.53			2.69			1.29
	June	0.58			0.12			2.85			1.16	0.99			3.16			1.60
	July	3.65			0.83			10.99	**	↑	2.02	0.21			0.05			2.98
	August	1.83			1.48			1.34			1.23	3.90	†	↑↑	0.01			0.75
	September	2.89			1.34			14.44	**	↑	0.52	0.01			1.83			0.79
	October	1.48			0.23			30.94	***	↑	2.84	1.59			1.12			0.46
P_{max}	May	7.31	*	↑	0.37			0.86			1.59	0.12			0.38			2.78
	June	0.03			1.00			10.13	**	↑	1.51	9.85	*	↑↑	3.13			2.02
	July	0.02			4.88	*	↓	1.81			0.11	4.64	*	↓↓	0.13			2.36
	August	1.69			5.75	*	↓	11.09	**	↓	0.30	0.85			1.04			0.19
	September	3.91			8.69	*	↑	0.68			0.64	0.44			6.87	*	↑↑	1.36
	October	8.59	*	↑	0.31			0.43			1.45	0.35			3.52	†	↑↑	0.13
J_{max}	May	ND		ND	ND			ND			ND	ND			ND			ND
	June	0.45			0.01			5.56	*	↑	1.16	7.00	**	↑↑	2.54			0.78
	July	0.36			6.96	*	↓	2.10			1.00	7.89	*	↓↓	0.32			0.25
	August	0.33			6.07	*	↓	9.85	*	↓	0.17	0.94			0.26			2.84
	September	0.15			6.19	*	↑	7.13	**	↓	2.73	0.00			7.02	*	↑↑	0.05
	October	12.00	*	↑	0.63			0.10			0.27	0.23			4.15	†	↑↑	0.11
V_{cmax}	May	ND			ND			ND			ND	ND			ND			ND
	June	0.24			1.38			4.86	*	↓	2.43	0.86			0.34			0.00
	July	0.67			1.66			6.04	*	↓	2.32	7.60	*	↓↓	2.15			3.32
	August	0.01			9.73	*	↓	4.76	†	↓	3.07	1.12			0.95			1.10
	September	0.00			9.02	*	↑	9.47	*	↓	0.11	0.71			0.03			0.03
	October	0.64			0.96			0.39			2.53	1.49			0.76			0.04
C/N	May	1.18			1.26			5.82	*	↑	0.04	0.72			0.03			0.50
	June	1.79			0.68			6.30	*	↑	0.04	0.39			1.07			0.03
	July	0.62			2.31			8.72	*	↑	0.53	0.44			0.54			2.48
	August	0.45			0.26			0.71			0.04	0.19			0.17			0.02
	September	0.72			17.93	***	↓	15.69	***	↑	1.03	0.89			1.23			0.06
	October	0.17			1.88			2.75			0.00	0.23			3.50	†	↓↓	0.00
$\delta^{13}C$	May	0.48			1.42			74.49	***	↓	0.01	7.17	**	↓↓	0.80			0.04
	June	1.31			0.01			152.62	***	↓	0.12	12.52	**	↓↓	4.39			0.82
	July	1.34			0.23			364.90	***	↓	0.79	1.34			9.45	**	↑↑	0.18
	August	6.41	*	↓	0.09			428.33	***	↓	1.00	5.59	**	↓↓	3.08			0.27
	September	6.08	*	↓	0.03			361.69	***	↓	1.96	0.63			2.49			0.41
	October	15.38	**	↓	0.03			332.44	***	↓	3.25	0.71			1.34			1.47

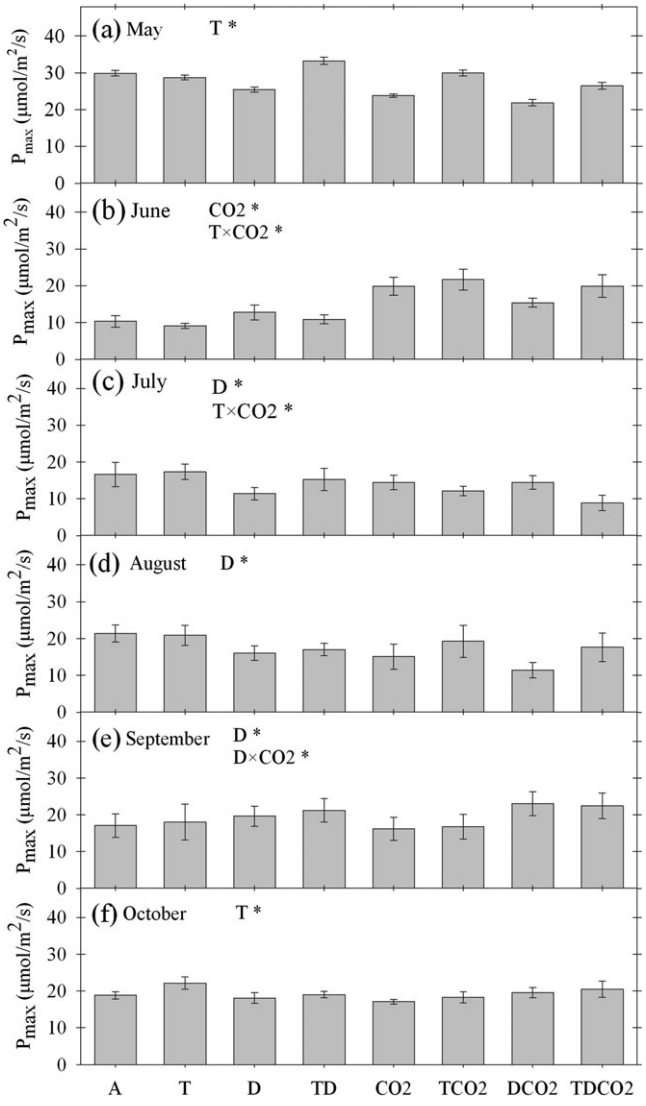

Fig. 6. Light- and CO_2-saturated net photosynthesis, P_{max}. Shown are the mean ±SE. Treatment abbreviations and statistics are as in Fig. 3.

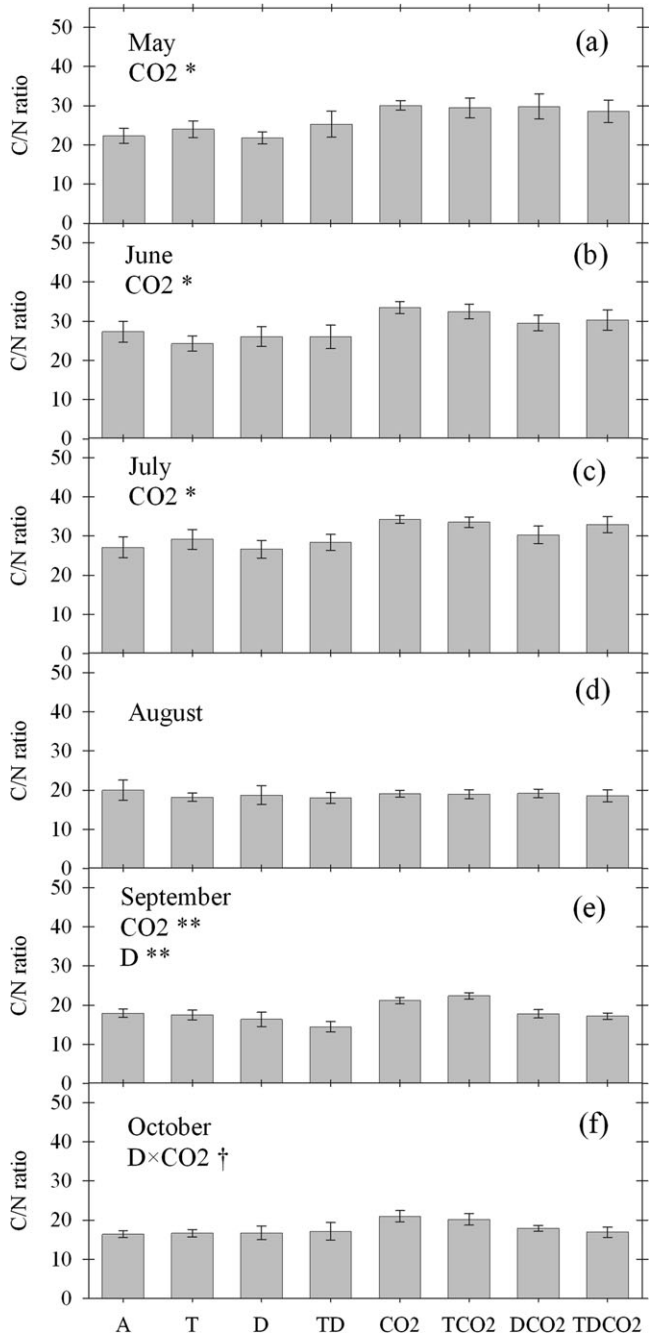

Fig. 7. Leaf carbon to nitrogen ratio, C/N. Shown are the mean ± SE. Treatment abbreviations and statistics are as in Fig. 3.

both opportunistic water consumption in *Deschampsia* and also that the up-regulation of photosynthetic capacity was the primary cause of the reduction of C_i/C_a.

It was hypothesized that elevated CO_2 would sustain P_n in dry periods via soil water savings; this is supported by the data obtained. Other studies report improved water relations in elevated CO_2 to originate mainly from g_s reductions in combination with LAI reductions (Körner, 2000; Volk *et al.*, 2000; Morgan *et al.*, 2004). In the present study no general g_s reductions were observed; other factors such as LAI reduction due to leaf wilting or the influence of co-occuring species could be proposed to explain the water savings that developed in the late part of the dry period in elevated CO_2 plots. The g_s of the co-occurring *C. vulgaris* decreased in response to dry conditions, but not as a general response to elevated CO_2, even when the soil water savings developed, partly excluding this option to explain the observed phenomenon (Albert *et al.*, 2011).

As reported in other climate change experiments, drought was the main factor determining grass biomass (Beier *et al.*, 2004; Penuelas *et al.*, 2004, 2007; Damgaard *et al.*, 2009; Prieto *et al.*, 2009*a*). In elevated CO_2, species-specific responses have been shown; some species increase biomass whereas other species remain unchanged (Ainsworth and Long, 2005; Maestre and Reynolds, 2007; Leakey *et al.*, 2009). This indicates that biomass reductions could have taken place in response to drought and that biomass could have been higher in elevated CO_2. Therefore, the opportunistic water consumption alone, or in combination with

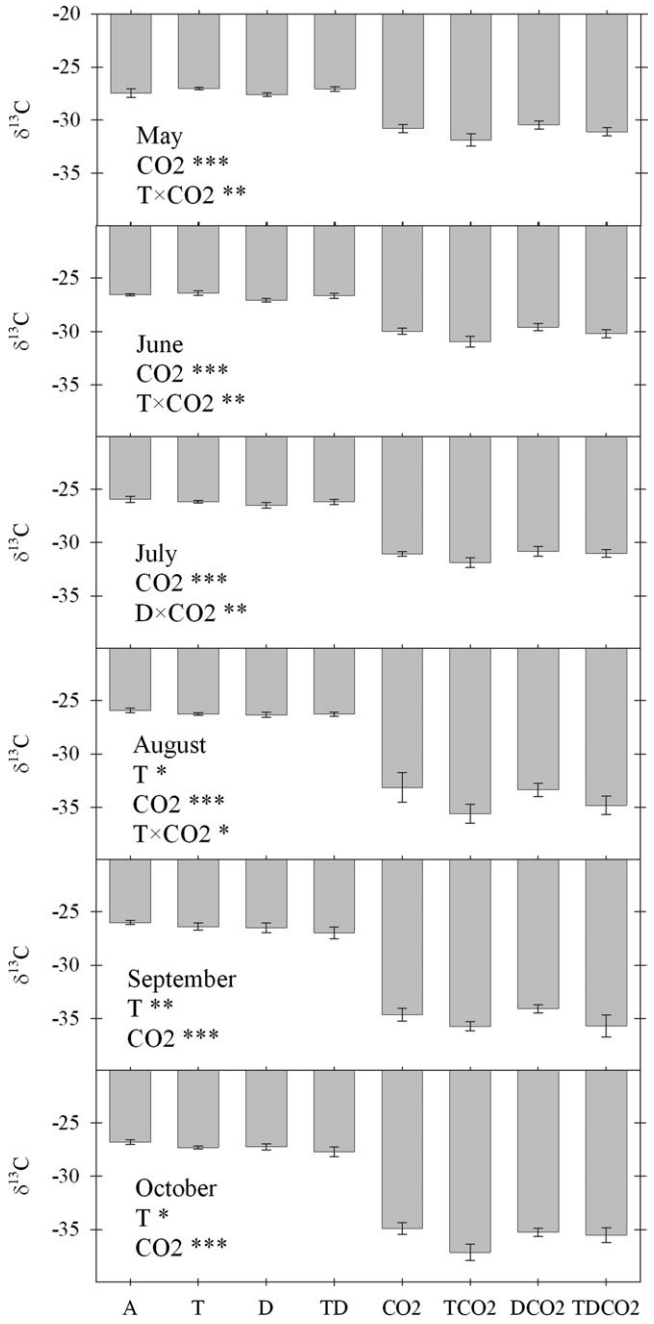

Fig. 8. Natural abundance of leaf ^{13}C, $\delta^{13}C$. Shown are the mean ±SE. Treatment abbreviations and statistics as in Fig. 3. Note that the CO_2 gas dosed in the FACE plots had a lower $\delta^{13}C$ signature of –10.6‰ $\delta^{13}C$ (May, June, and July) and of –13.8‰ $\delta^{13}C$ (August, September, and October) as opposed to –8.0‰ in ambient air. This caused the significantly lower $\delta^{13}C$ values in leaves in the elevated CO_2 treatment.

high biomass, probably led to depletion of soil water to a threshold near the wilting point in elevated CO_2 in late July. Thereafter, in response to the very low July soil water availability, g_s was at its seasonal low and above-ground grass biomass probably died back. Consequently, this leaf wilting left the g_s in the remaining leaves unaffected, but P_n was stimulated. This complex balance between the opportunistic water consumption and drought-induced leaf wilting of *Deschampsia* caused the modest soil water savings observed under elevated CO_2. Such response patterns provide further support for the concept that limitations on photosynthetic improvement in elevated CO_2 are governed by water relations, as shown by several other FACE experiments (Körner, 2000; Volk *et al.*, 2000; Morgan *et al.*, 2004; Leuzinger and Körner, 2007), but the opportunistic water consumption and variable grass biomass complicate the picture and need further investigation.

Responses to passive night-time warming and elevated CO_2 in combination

In accordance with the hypothesis proposed herein, warming improved photosynthetic capacity (P_{max} and J_{max}) in the early and late season, although, contrary to this hypotheses, this did not affect P_n. This could be due to higher leaf respiration, since leaf respiration increases with temperature (Atkin and Tjoelker, 2003). However, it does clearly indicate an earlier maturation of photosynthetic capacity in response to warming, which is in line with other studies (Penuelas and Fiellala, 2001; Cleland *et al.*, 2006; Menzel *et al.*, 2006; Prieto *et al.*, 2009*b*) showing an advancement of the growing season. Warming also appeared to shift the timing of the second phase leaves as $\delta^{13}C$ was reduced in warmed plots in August, September, and October. In this period, water supply was sufficient; there were no significant effects of warming on P_n or g_s. This indicates that the effect of warming on $\delta^{13}C$ was caused by an earlier leaf emergence allowing for a longer period of carbon uptake and thus discrimination against the ^{13}C.

The effects of warming and elevated CO_2 were not additive as hypothesized; T×CO2 synergistically increased P_n in parallel with T×CO2 synergistically decreasing $\delta^{13}C$ (May and June). Further, T×CO2 synergistically increased June P_{max} and J_{max}, but in July this reversed. These responses clearly demonstrate that T×CO2 increases plant carbon uptake early in the growing season and that this is closely related to the regulation of photosynthetic capacity. This shows that earlier maturation of photosynthetic capacity in response to warming is a prerequisite for the interactive stimulation of P_n when combined with elevated CO_2, early in the growing season.

After rewetting, the T×CO2 interaction synergistically increased August P_n and decreased $\delta^{13}C$, but this increased plant carbon uptake was not governed by photosynthetic capacity regulations. While the warming treatment resulted in an extended period of carbon uptake (see above), this cannot explain the profound short-term stimulation of P_n. Therefore, it is suggested that the T×CO2 stimulation of P_n could be linked to better access to soil water mediated by extended root growth, facilitated by the previous month's high carbon uptake in the plots with both warming and elevated CO_2. Alternatively, it may be the result of a complex mechanism facilitating enhanced export of photoassimilates to sink tissues via increased night-time respiration in response to warming (Turnbull *et al.*, 2002, 2004) or direct stimulation of leaf respiration by elevated

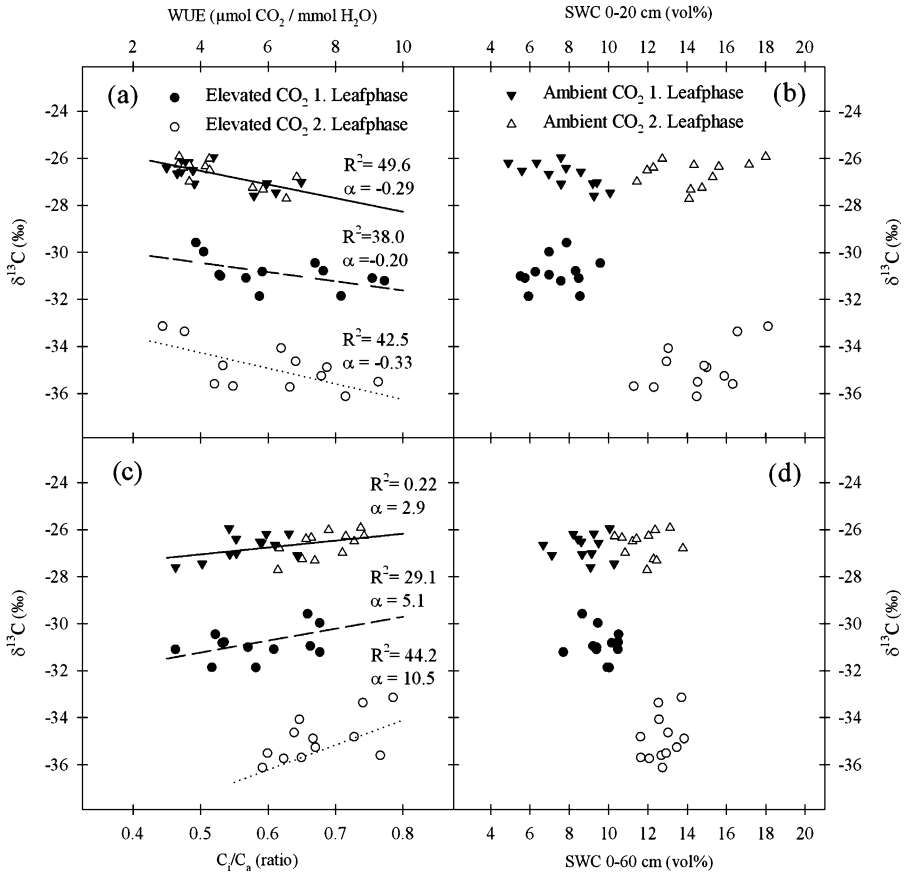

Fig. 9. Regressions with leaf carbon isotope ratio ($\delta^{13}C$). (a) The leaf $\delta^{13}C$ versus water use efficiency. (b and d) the leaf $\delta^{13}C$ versus the soil water content during each of the leaf gas exchange campaigns at 0–20 cm and 0–60 cm. (c) The leaf $\delta^{13}C$ versus the ratio between the intercellular CO_2 concentration and the CO_2 concentration outside the leaf. Points are the averages from each treatment per month. Leaves from the primary leaf phase (May, June, and July) are indicated by filled symbols and leaves from the secondary leaf phase (August, September, and October) by open symbols. Circles are elevated CO_2 (FACE) and triangles ambient CO_2 (non-FACE). Note the average air $\delta^{13}C$ during the primary and secondary leaf phase under elevated CO_2 was –10.6‰ and –13.8‰, respectively. In ambient CO_2 the average air $\delta^{13}C$ was –8‰. This caused the offsets in leaf $\delta^{13}C$ between the leaf phases and ambient versus elevated CO_2. Only significant regression lines with slope (α) different from zero are depicted (P <0.05).

CO_2 (Leaky *et al.*, 2009*b*). In a parallel study at the experimental site the grass biomass was synergistically increased in response to the combination of warming and elevated CO_2 (Andresen *et al.*, 2009). These responses demonstrate that T×CO2 synergistically increased plant carbon uptake to be strongly influenced by photosynthetic capacity up-regulation early in the season. After rewetting, the processes are less clear, but mechanisms stimulating the carbon sink strength in the combination of elevated CO_2 and warming could be involved.

Responses of drought and elevated CO_2 in combination

In accordance with the hypothesis proposed herein, the experimental drought decreased P_n via g_s reduction (July), but also via photosynthetic capacity (P_{max}, J_{max}, and V_{cmax}) down-regulation under severe water shortage (August). Gradually drier conditions in general reduce above-ground *Deschampsia* biomass, and the experimental drought treatment also reduced the vegetation coverage (J. Kongstad *et al.*, personal communication). These responses demonstrate the intensity of the experimental drought to push the response of *Deschampsia* beyond the threshold where dry conditions could be handled solely by acclimation, and leaf wilting occurred.

During dry conditions in the combination of elevated CO_2 and drought, there were no indications of SWC being higher than in the control. Although a D×CO2 interaction did increase the SWC above what was expected from the single factors, the resulting SWC was relatively lower in the DCO2 plots compared with controls, but above the level in the D plots (Figs 2, 3). The absence of beneficial water savings was reflected in the D×CO2 interaction inducing the higher $\delta^{13}C$ in DCO2 and TDCO2 plots. In other grassland studies, elevated CO_2 has resulted in soil water savings (Bremer *et al.*, 1996; Niklaus *et al.*, 1998; Morgan *et al.*, 2004), mainly brought about by g_s reduction along with higher potential to sustain P_n under dry conditions (Ainsworth and Long, 2005; Lauber and Körner, 1997; Robredo *et al.*, 2007). As discussed above, such g_s reductions were not seen in

elevated CO_2 to counteract the negative influence of the drought. Furthermore, the experimental drought caused very strong negative effects on photosynthetic performance, explaining why the experimental drought responses seem to dominate the physiological responses in the DCO2 and TDCO2 treatments.

Rewetting more than reversed all negative ecophysiological effects induced by the drought. After the experimental drought treatment, P_n, g_s, P_{max}, and J_{max} increased and the C/N ratio decreased. In addition, the D×CO2 interaction synergistically increased the photosynthetic capacity (P_{max} and J_{max}) mediating parallel stimulatory effects on P_n in September and October. These responses to rewetting indicate that better nitrogen availability, perhaps in combination with pronounced reallocation from rhizomes, does control the magnitude of P_n beyond the drought period. Increased nitrogen availability could also explain why the C/N ratio did not increase in elevated CO_2 in the autumn even though P_n was increased. These variable responses show that P_n, in the combination of drought and elevated CO_2, depends on soil water availability, with additive effects on P_n when SWCs are low and synergistic D×CO2 effects on P_n after rewetting.

Influence of T×CO2 and D×CO2 interactions on carbon uptake is controlled by photosynthetic capacity and plant available water

The heathland ecosystem investigated can be considered to be fully developed, with the LAI and fine root renewal at steady state, and can as such be compared with mature forest ecosystems as conceptualized by Körner (2006). In such a system, the stimulation of biomass production may to a large degree result from CO_2-induced improved water relations and to a lesser degree directly from elevated CO_2. The *Deschampsia* responses in the present study add complexity to this concept as the per leaf area water consumption was opportunistic and the biphasic growth pattern allowed for leaf dieback at low soil water availability and rapid re-growth of new active leaves when rewetted. This growth characteristic with an opportunistic resource exploration allowed for photosynthetic capacity regulations that mediated the synergistic T×CO2 and D×CO2 interaction effects on photosynthesis. These characteristics are advantageous when exposed to climatic changes, and the carbon uptake of this species is likely to be strongly influenced by changes in precipitation patterns, such as event frequency, intensity, magnitude, and interannual variability. More long-term experimentation including years with different variability in precipitation and the warming impact at the shoulders of the growing season is needed to elaborate these findings. In conclusion, after 1 year of experimentation the limitations imposed by low soil water availability and stimulation in the early and late season by warming clearly structure and interact with the photosynthetic response to elevated CO_2 in this grassland species.

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